

EP 0217768

APR 1987

BEST AVAILABLE COPY

<p>87-095711/14 B04 D16 J04 S03 YINE/ 05.09.85 YINET *EP -217-768-A 05.09.85-US-772846 (08.04.87) C12q-01/56 G01n-33/86 Heparin determ. in blood plasma sample - using admixture contg. calcium chloride, brain phospholipid(s) and buffered plasma fraction C87-039793 E(AT DE FR GB IT NL SE)</p>	<p>B(4-B1B, 4-B4D3, 4-B4D4, 4-C2E1, 5-A1B, 11-C8E, 12-K4A2) D(5-H9) J(4-B1B) 7</p>
<p>Determn. of the amt. of heparin (I) in a blood plasma sample comprises (a) incubating the sample for a limited period of time with a known amt. of factor Xa, which known amt. is in excess of the amt. needed to react with all (I) - at III (antithrombin III) complexes in the blood plasma sample within a given time period, (b) combining the incubated prod. with an admixture of (1) calcium chloride, (2) brain phospholipids and (3) a buffered plasma fraction which has been produced by treating mammalian blood to remove clotting factors II, VII, IX and X while retaining clotting factor V and fibrinogen, the buffered admixture being characterised in that (i) it does not clot by itself for at least 24 hrs. at 37°C, (ii) it forms a firm clot in the presence of added thrombin.</p>	<p>(iii) it contains at least 50% of Factor V which is present in 1 ml of normal human plasma and (iv) it provides a linear heparin dilution curve using a standard heparin prepn., and (c) measuring the time it takes for clotting to occur after combining the incubated prod. of step (a) with the buffered admixture, which clotting time is directly proportional to the concn. of (I) present in the blood plasma sample. The assay compsn. in (b) is also claimed. <u>USE/ADVANTAGE</u> The determn. of (I) provides an important parameter for the supervision of heparin treatment which is often administered in the presence of a threat of thrombosis. The method is an improved, simplified version of the prior art method. <u>REAGENT</u> The plasma fraction of (b) (3) can be prepd. by (a) treating mammalian blood with an anticoagulant and then removing blood cells to produce a plasma, (b) subjecting the plasma to a sepn. step to remove EP-217768-A+</p>

© 1987 DERWENT PUBLICATIONS LTD.

128, Theobalds Road, London WC1X 8RP, England

US Office: Derwent Inc. Suite 500, 6845 Elm St. McLean, VA 22101

Unauthorised copying of this abstract not permitted.

coagulation Factors II, VII, IX and X,

(c) subjecting the plasma to a sepn. step to recover a protein fraction contg. fibrinogen and coagulation Factor V,

(d) treating the protein fraction to remove soluble ammonium salts and insoluble proteins and

(e) buffering the clear plasma fraction obtd.

**EXAMPLE**

9 l of bovine blood was anticoagulated with 1 l of 3.8% sodium citrate soln. then centrifuged at 2500g. To 1 l. of the plasma obtd. was added 7.35 g followed by 20.825g of solid  $\text{BaCl}_2$  over 90 mins. The ppt. was removed by centrifugation at 3000g for 20 mins. 270g of solid  $(\text{NH}_4)_2\text{SO}_4$  was added and the plasma allowed to set for 90 mins. and then centrifuged at 4000g for 15 mins. The recovered pptd. protein fraction was dissolved in 350 ml of water and dialysed against 0.9 M NaCl soln. until all ammonium ions had been removed.

The fraction was then centrifuged at 3000g for 15 mins. and buffered to pH 7.5 by mixing 9 vols. with 1 vol. of a soln. contg. 2.5g of polyethylene glycol, 90g of NaCl and 98.88g of tris-maleate in 1 l of water.  $\text{CaCl}_2$  was added to a concn. of 0.025M and cephalin was added at 1 vol. % to obtain admixture (A).

At 37°C, 0.1 ml of an undiluted test plasma was pipetted into a tube. To it was delivered 0.1 ml of Factor Xa with mixing. 120 Secs. after the addn., 0.1 ml of admixture (A) was added. The clotting time was observed to be 45 secs. which a calibration curve indicated meant a heparin content of 0.15 units/ml of plasma. (18pp1703HDDwgNo0/1).  
(E) ISR: No Search Report.

EP-217768-A

© 1987 DERWENT PUBLICATIONS LTD.

128, Theobalds Road, London WC1X 8RP, England

US Office: Derwent Inc. Suite 500, 6845 Elm St. McLean, VA 22101

*Unauthorised copying of this abstract not permitted.*

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets

(11)

Publication number:

**0 217 768**  
**A2**

(12)

## EUROPEAN PATENT APPLICATION

(21)

Application number: 86850288.1

(51)

Int. Cl.<sup>4</sup>: **G 01 N 33/86**  
**C 12 Q 1/56**

(22)

Date of filing: 02.09.86

(30)

Priority: 05.09.85 US 772846

(43)

Date of publication of application:  
08.04.87 Bulletin 87/15

(84)

Designated Contracting States:  
AT DE FR GB IT NL SE

(71)

Applicant: Yin, E. Thye  
2335 S. Hanley Road  
St. Louis Missouri 63144(US)

(72)

Inventor: Yin, E. Thye  
2335 S. Hanley Road  
St. Louis Missouri 63144(US)

(74)

Representative: Fogelberg, Lennart et al,  
STENHAGEN PATENTBYRA AB Karlavägen 18  
S-114 31 Stockholm(SE)

(54)

Method and compositions for heparin assays.

(57)

This invention pertains to an improved method for determining the amount of heparin in a blood sample, and compositions useful in connection with the method.

EP 0 217 768 A2

## METHOD AND COMPOSITIONS FOR HEPARIN ASSAYS

This invention pertains to an improved method for determining the amount of heparin in a blood sample, and compositions useful in connection with the method.

5

### BACKGROUND

The determination of heparin establishes an important parameter for the supervision of heparin treatment which is often administered in the presence of a threat of thrombosis. Heparin forms with antithrombin III (AT III) a complex which inhibits the proteolytic activity of thrombin. Since thrombin catalyzes the formation of fibrin from fibrinogen, the thrombin activity is responsible for the coagulation of blood or plasma and hence also for the fibrin clots which form in thrombosis. Heparin treatment is often applied in the presence of a threat of thrombosis (e.g. before surgical interventions). A precise adjustment of heparin concentration is therefore extremely important. If the dose is too low, there is the danger of thrombosis or embolism, which can result in death. Excessively high heparin concentrations, however, result in bleeding. The quantitative analysis of heparin, therefore, is one of the tests most frequently performed in the blood testing laboratory.

In 1973 Yin and co-workers developed the first quantitative assay method for the in vitro measurement of heparin in plasma based on Factor Xa neutralization (J. Lab. Clin. Med. 81: 298, 1973). This assay method had the advantage over the methods known prior to 1973 in that it could detect less than 0.02 units of heparin per milliliter of plasma. However, the assay is cumbersome and time consuming to perform. It is a two-stage assay, and requires much manual manipulation. In the first-stage of the assay, the patient's plasma sample is incubated with normal plasma, buffer, and a known excess of Factor Xa for a predetermined time period, after which a sub sample from this primary reaction mixture is removed and assayed for Factor Xa activity. The latter step constitutes the second-stage of the assay. Factor Xa activity is measured by the addition of the test sample to another test tube, and to it calcium chloride, cephalin in bovine plasma are added separately in timed fashion. A commercial embodiment of this method is described in detail in Sigma Chemical Company Technical Bulletin No. 870 (as revised in January 1982). The disclosure of this bulletin is incorporated herein by reference.

#### THE PRESENT INVENTION

I have now developed an improved, simplified version of the above identified Yin et al method and the improved method involves (a) incubating an undiluted patient blood plasma sample with an amount of Factor Xa which exceeds that which can be completely neutralized by the heparin in the patient blood

sample (b) contacting the incubated product with a reagent containing an admixture of calcium chloride, phospholipids (e.g. brain cephalin) and a specially prepared buffered blood plasma fraction to thereby develop the appearance of a clot and (c) comparing the clotting time in step (b) with the clot times on an established calibration curve obtained by plotting clotting time versus heparin concentration to thereby determine the amount of heparin in the patient blood plasma sample.

Considered from one aspect the present invention involves a method for determining the amount of heparin in a blood plasma sample, which method comprises

- (A) incubating the blood plasma sample for a fixed period of time with a known amount of Factor  $X_a$ , which known amount is in excess of the amount needed to react with all the heparin - at III complexes in the blood plasma sample,
- (B) combining the incubated product of step (A) with an admixture of

- (1) calcium chloride,
- (2) brain phospholipids, and
- (3) a buffered plasma fraction that has been produced by treating mammalian blood to substantially remove clotting Factors II, VII, IX and X while retaining clotting Factor V and fibrinogen,

said admixture being characterized by the fact that

- (a) it does not clot by itself for at least 24 hours at 37°C, and
- (b) it forms a firm clot in the presence of added thrombin, and

- (c) it contains at least 50% of Factor V that is present in one ml of normal human plasma, and
- (d) it provides a linear heparin dilution curve using a standard heparin preparation, and

5 (C) measuring the time it takes for clotting to occur after  
5 combining the incubated product of step (A) with said  
buffered admixture, which time is directly proportional  
to the concentration of heparin present in the blood plasma  
sample.

10 The method is suitably carried out with 100 microliters.  
10 of a patient's blood plasma, 100 microliters of Factor  $X_a$  and  
100 microliters of the admixture set forth in (B) above.

The Factor  $X_a$  can be prepared from bovine plasma by any  
method described in the literature. For example, Yin, E.T.,  
15 Wessler, S., and Stoll, P., J. Biol.Chem.243: 112 (1968). The  
1! purified Factor  $X_a$  is further lyophilized and stabilized in a  
buffer containing crystalline bovine serum albumin, polyethylene  
glycol, NaCl, and Tris (hydroxymethyl) aminomethane maleate,  
pH 7.5. Factor  $X_a$  as described in the aforementioned Sigma-  
20 Bulletin No. 870 can be used.

2 Incubation of the patient's blood plasma and Factor  $X_a$   
preferably takes place at 37°C for 60 - 160 seconds, and more  
preferably for a time period of 120 seconds.

The plasma fraction of (B) (3) is mammalian blood that is  
25 substantially free of Factors II, VII, IX and X but which still  
2 contains Factor V and fibrinogen, and can be produced by the  
steps of

- (i) treating mammalian blood with an anticoagulant and then removing blood cells to produce a plasma,
- (ii) subjecting the plasma from step (i) to at least one separation step to remove coagulation Factors II, VII, IX and X, and recovering the resulting plasma,
- (iii) subjecting the plasma resulting from step (ii) to at least one further separation step and recovering therefrom a protein fraction containing fibrinogen and coagulation Factor V, and
- (iv) treating the protein fraction recovered from step (iii) to remove therefrom soluble ammonium salt and insoluble proteins and recovering a clear plasma fraction, and
- (v) buffering the clear plasma fraction obtained from step (iv) so as to obtain a buffered plasma fraction.

The buffered plasma fraction produced by steps (i) - (v) is admixed with calcium chloride and brain phospholipids and the resulting admixture has the characteristics (a) - (d) set forth above.

In step (i) 9 volumes of the mammalian blood can be treated with one volume of 3.8% sodium citrate solution to effect anticoagulation and then centrifuged at 2500xg to remove blood cells. Bovine blood is preferred.

In step (ii) the separation can be effected by adding to 1000 ml of plasma from step (i) 7.35 gm of trisodium citrate, stirring to dissolve the citrate, followed by the addition of 20.825 gms of barium chloride (sufficient to achieve an overall barium chloride concentration of about 0.1M in the plasma mixture) at room temperature over a period of 1-2 hours, and the heavy precipitate thus formed with the aid of barium citrate can be removed by the centrifugation of the plasma mixture at 3000x g for 20 minutes (the heavy precipitate containing Factors II, VII, IX and X) leaving a clear plasma supernatant.



Instead of using citrate blood and adsorbing the Factors II, VII, IX and X with a barium-citrate salt, one could employ aluminum hydroxide, or barium sulfate, or any other method which will remove these clotting proteins, e.g. ion exchanger column chromatography. If barium sulfate is used blood collected in sodium or potassium oxalate is used instead of sodium citrate.

In step (iii) the separation can take the form of fractionation with solid ammonium sulfate at a concentration of approximately 270 gm per liter of the plasma at room temperature. If the resulting plasma mixture is allowed to sit at room temperature for 1 - 2 hours, and then centrifuged at 4000 x g for 15 minutes at room temperature, a precipitated protein fraction can be obtained. The concentration of ammonium sulfate of 270 gm/l corresponds to a 40 - 45% saturated solution. At a salt concentration of about 30% saturation fibrinogen precipitates out; as the salt concentration increases above 30% saturation to the area of 40% saturation the Factor V proteins precipitate out, as well as some other as yet unidentified proteins. Thus, whereas I have found that an ammonium sulfate concentration of 270 gm/l is quite suitable in precipitating out fibrinogen and Factor V proteins, I feel that lowering or increasing this concentration would still result in a quite satisfactory protein fraction. The use of ammonium sulfate to precipitate fibrinogen is described in a number of publications, e.g. "The Biochemistry of Blood Coagulation" by T. Astrup, in *Acta Physiologica Scandinavica*, Supplement 21, 1944. The use of ammonium sulfate for the fractionation of Factor V is also described in a number of publications, e.g. the publication "Human Blood Coagulation and Its Disorders" edited by Biggs & MacFarlane, p. 54, 3rd edition 1962.

Instead of using ammonium sulfate to precipitate Factor V and fibrinogen, equivalent separation might also be achieved with alcohol or column chromatography on ion exchangers, or gel filtration based on molecular size sieving principles.

5 In step (iv) the protein precipitate from step (iii) is harvested and dissolved in a volume of distilled water equal to 30 - 40% of the original plasma volume. This "plasma fraction" is then dialysed against 0.9% NaCl solution until no ammonium ion is present in the fraction. The dialysed plasma fraction is clarified by  
10 centrifugation at 3000 x g for 15 minutes at room temperature.

In step (v) the clear plasma fraction from step (iv) can be buffered to a pH of 7.5 by mixing nine volumes of it with one volume of a solution containing 2.50 gm polyethylene-glycol, 90.0 gm. NaCl, 98.88 gm. Tris-maleate per liter of  
15 distilled water.

To the buffered-plasma fraction resulting from steps (i) - (v) calcium chloride is added to a final calcium chloride concentration of 0.025M., followed by the addition of brain phospholipids, such as the cephalin preparation of Bell, W.N. and Alton, H.G., Nature, 174:880(1954). The amount of cephalin  
20 required to be added to the "plasma fraction" will depend on the concentration of Factor X<sub>a</sub> chosen for the assay. For example, the more concentrated the Factor X<sub>a</sub> is in the assay system, the less amount of cephalin is needed, and vice versa.

If calcium chloride and phospholipid solutions are mixed together they will ordinarily quickly precipitate. In accordance with the present invention it has been discovered that precipitation can be avoided if these substances are mixed together in the presence of the buffered plasma fraction that results from  
25 steps (i) - (v). The resulting admixture is characterized by

the fact that

- (a) it does not clot by itself for at least 24 hours at 37°C, and
- (b) it forms a firm clot in the presence of added thrombin, and
- (c) it contains at least 50% of Factor V that is present in one ml of normal human plasma, and
- (d) it provides a linear heparin dilution curve using a standard heparin preparation.

10

I have found it convenient to collect a blood specimen by clean venepuncture in a plastic tube containing 3.8% sodium citrate. The ratio of blood to anticoagulant is 9:1. The citrated blood is then centrifuged within two hours of collection at 4000 x g for 20 minutes, at +5 to +20°C. The platelet-poor plasma is carefully removed and either stored for up to 24 hours at +5°C or frozen if it is not to be tested immediately.

15

1

For manual testing the equipment that is recommended includes 12 x 75 mm glass test tubes, pipettes to deliver 0.1 ml, distilled water, two stopwatches and a 37°C water bath. If a clot detector machine is to be used, such as a BBL Fibrometer, a 0.3 ml probe is employed.

20

Prior to testing it has been found desirable to

- (a) prewarm a convenient volume of said admixture (B) for at least 5 minutes at 37°C and it can be left at this temperature for up to 2 hours,
- (b) prewarm the plasma to be tested at 37°C for 1 - 10 minutes,

25

- (c) prewarm the clotting vessels (e.g. the test tubes or fibrocups) at 37°C,
- (d) maintain the Factor X<sub>a</sub> solution at room temperature (25°C) for up to 8 hours.

5 As noted above, it has been found preferable to carry out the assay at 37°C ± 0.5°.

The three steps that are followed in the usual assay include:

Step 1 - pipetting 0.1 ml of undiluted test plasma into a clotting tube,

10 Step 2 - delivering 0.1 ml Factor X<sub>a</sub> to Step 1, and simultaneously starting the first stopwatch, mixing well,

Step 3 - Exactly 120 seconds after the addition of Factor X<sub>a</sub> in Step 2, adding 0.1 ml of the admixture used in (B) above and simultaneously starting the second stopwatch.

5 The number of seconds it takes for the above mixture (steps 1 - 3) to form a solid clot is noted and can be converted to units of heparin per ml of plasma using a standard heparin calibration curve such as is shown in Figure 1. The methods for preparing such calibration curves are well known in the art (e.g. the aforementioned Sigma Technical Bulletin 870).

The following examples will serve to illustrate the invention:

EXAMPLE 1

Nine liters of bovine blood was anticoagulated with one liter of 3.8% sodium citrate solution and then centrifuged at 2500 x g to remove blood cells. To one liter of the resulting plasma was  
5 added 7.35 gms of trisodium citrate, which was stirred to dissolve the citrate, followed by the addition of 20.825 gms of solid barium chloride at room temperature over a period of about 90 minutes. The heavy precipitate which formed was removed by centrifugation of the plasma mixture at 3000 x g for about 20  
10 minutes leaving a clear plasma supernatant. 270 g of solid ammonium sulfate was added to the clear plasma supernatant at room temperature and allowed to set for 90 minutes and then centrifuged at 4000 x g for 15 minutes, and a precipitated protein fraction obtained. The recovered precipitated protein  
15 fraction was dissolved in 350 ml of distilled water and then dialysed against a 0.9 NaCl solution until all ammonium ions had been removed. The dialysed plasma fraction was then clarified by centrifugation at 3000 x g for 15 minutes at room temperature and then buffered to a pH of 7.5 by mixing nine  
20 volumes of it with one volume of a solution containing 2.5 gm of polyethylene glycol, 90.0 gm of NaCl, 98.88 gm of tris-maleate, dissolved in 1 liter of distilled water. Calcium chloride was added to this buffered plasma fraction to achieve a calcium chloride concentration of .025M, whereafter cephalin [prepared  
25 by Bell and Alton, Nature, 174:880 (1954)] was added in a volume ratio of one volume of such cephalin to 99 volumes of the buffered plasma fraction to which calcium chloride had already been added. The resulting admixture served as one of the assay materials for my method and Factor X<sub>a</sub> served as the other  
30 assay material for my method.

In an environment maintained at 37°C 0.1 ml of an undiluted test plasma containing an unknown amount of heparin was pipetted into a clotting tube. To it was delivered 0.1 ml of Factor X<sub>a</sub> (obtained by the aforementioned method of Yin et al) with mixing and at the time of delivery a stopwatch was started. Exactly 120 seconds after said addition of Factor X<sub>a</sub>, 0.1 ml of the admixture of Example 1 was added and a second stopwatch was started. The clotting time was observed to be 45 seconds which a calibration curve indicated meant a heparin content of .15 units/ml of plasma.

### EXAMPLE 3

The procedure of Example 2 was followed except that the Factor X<sub>a</sub> used was Activated Factor X Reagent, Stock No. 870-10 from the Sigma Chemical Company of St. Louis, Missouri. Results comparable to that of Example 2 were obtained.

In order to decrease the clotting time in Example 2 the concentration of cephalin in Example 1 can be increased or the Factor X<sub>a</sub> concentration in Example 2 can be increased.

I contemplate that my invention can be made available in kit form that would comprise in a freeze-dried form, (A) a container of Factor X<sub>a</sub> and (B) a container of an admixture of calcium chloride, brain phospholipids and a buffered plasma fraction that has been produced by treating mammalian blood to substantially remove clotting factors II, VII, IX and X while retaining clotting factor V and fibrinogen. The components (A) and (B) are preadjusted so that when employed in the assay they will provide optimal sensitivity to the heparin in blood plasma samples.

- 12 -

Whereas my invention has been specifically discussed in connection with heparin, it should also be understood that the invention is also applicable to heparin-like compounds and it is therefore to be understood that the term "heparin" herein includes heparin and heparin-like compounds whether they be natural, synthetic, high or low molecular weight heparin fractions or fragments.

1. A method for determining the amount of heparin in a blood plasma sample which comprises

5 (A) incubating the blood plasma sample for a limited period of time with a known amount of factor  $X_a$ , which known amount is in excess of the amount needed to react with all the heparin - at III complexes in the blood plasma sample within a given time period,

10 (B) combining the incubated product of step (A) with an admixture of

(1) calcium chloride,

(2) brain phospholipids, and

15 (3) a buffered plasma fraction that has been produced by treating mammalian blood to substantially remove clotting factors II, VII, IX and X while retaining clotting factor V and fibrinogen,

- said buffered admixture being characterized by the fact that

20 (a) it does not clot by itself for at least 24 hours at 37°C, and

(b) it forms a firm clot in the presence of added thrombin, and

(c) it contains at least 50% of Factor V that is present in one ml of normal human plasma, and

(d) it provides a linear heparin dilution curve using a standard heparin preparation, and

measuring the time it takes for clotting to occur after combining the incubated product of step (A) with said buffered admixture, which clotting time is directly proportional to the concentration of heparin present in the blood plasma sample.



2. An assay composition comprising an admixture of

(1) calcium chloride,

(2) brain phospholipids, and

(3) a buffered plasma fraction that has been produced by treating mammalian blood to substantially remove clotting factors II, VII, IX and X while retaining clotting factor V and fibrinogen,

- said admixture being characterized by the fact that

(a) it does not clot by itself for at least 24 hours at 37°C, and

(b) it forms a firm clot in the presence of added thrombin, and

(c) it contains at least 50% of factor V that is present in one ml of normal human plasma, and

(d) it provides a linear heparin dilution curve using a standard heparin preparation.

3. A kit for determining the amount of heparin in a blood plasma sample which comprises

(A) a container of factor  $X_a$ , the amount of the factor  $X_a$  being in excess of the amount needed to react with all the heparin

- at III complexes in a selected blood sample within a given time period,

(B) a container of an admixture of

(1) calcium chloride,

(2) brain phospholipids, and

(3) a buffered plasma fraction that has been produced by treating mammalian blood to substantially remove clotting factors II, VII, IX and X while retaining clotting factor V and fibrinogen,

-said admixture being characterized by the fact that

does not clot by itself for at least 24 hours  
at 37°C, and  
it forms a firm clot in the presence of added  
thrombin, and

5 Beyond (1) it contains at least 50% of factor V that is  
present in one ml of normal human plasma, and  
(2) it provides a linear heparin dilution curve  
using a standard heparin preparation.

10 4. A method for producing an assay composition comprising

- (a) treating cell-free mammalian blood plasma to remove Factors  
II, VII, IX and X and recovering a clear plasma supernatant,
- (b) subjecting said clear plasma supernatant to a protein  
fractionation treatment and recovering a protein fraction  
15 that contains fibrinogen and Factor V,
- (c) subjecting the protein fraction of step (b) to a purification  
treatment to remove therefrom any soluble ammonium salt and  
insoluble proteins, and obtaining a clear plasma fraction,
- (d) buffering said clear plasma fraction to obtain a buffered  
20 plasma fraction,
- (e) admixing said buffered plasma fraction with calcium chloride  
and brain phospholipids, and recovering an admixture that  
is characterized by the fact that

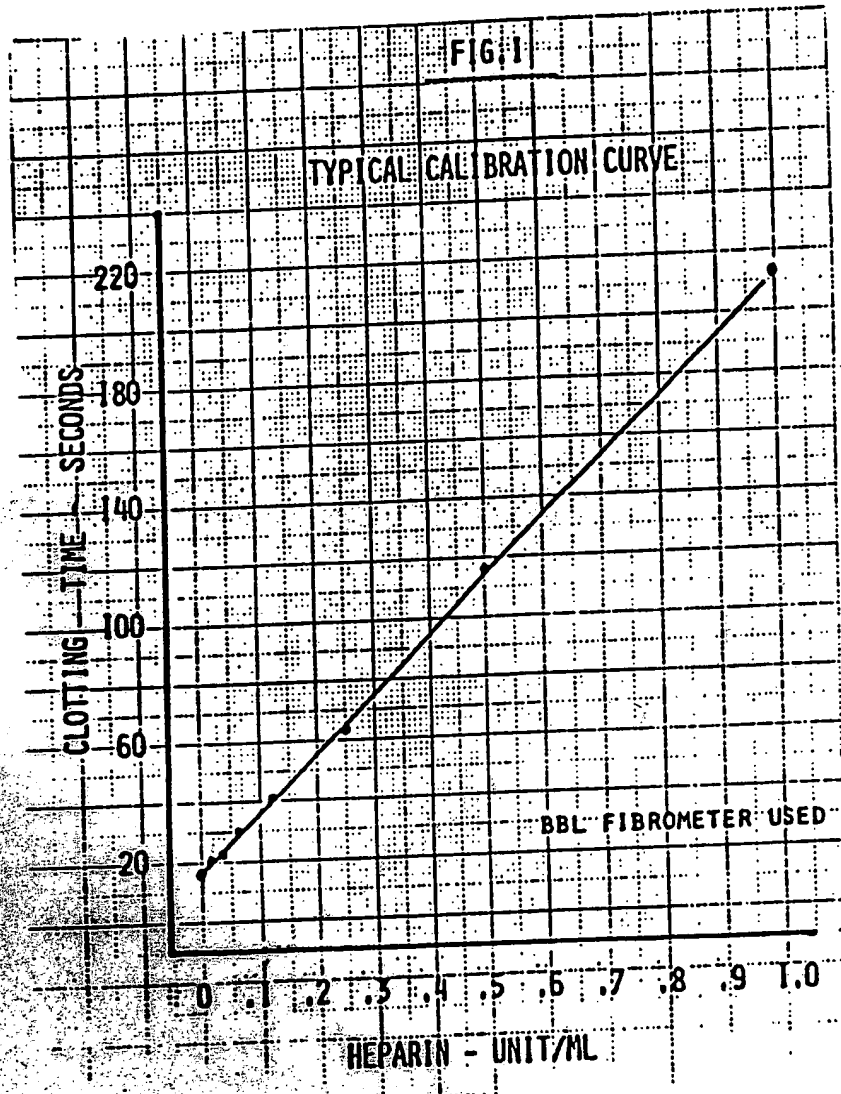
(1) it does not clot by itself for at least 24 hours at  
25 37°C, and

(2) it forms a firm clot in the presence of added thrombin,  
and

it contains at least 50% of factor V that is present  
in one ml of normal human plasma, and

(4) it provides a linear heparin dilution curve using a standard heparin preparation.

5. A kit according to Claim 3 wherein the reagent in each of said two containers is preadjusted so that when employed in an assay they will provide optimal sensitivity to the heparin present in blood plasma samples.



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**